Acknowledgments

We wish to thank Richard Getz, Albert Brown, Peter Pappas, and Spencer Farr for their participation in this project.

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Introduction

The composition of tobacco and tobacco smoke have been the subject of intensive research. This paper presents evidence that ROMAR™, an inexpensive, non-toxic, commercially available colloidal compound, can adsorb and thus remove tar and harmful agents from cigarette smoke *without a change in the draw or rate of the modified cigarette.*

Conventional methods for removing or reducing undesirable chemical components from the tobacco smoke include the use of filters consisting of cellulose acetate fiber, activated carbon, or the like. These filters reduce the concentration of such components in tobacco smoke to an extent, but their efficiency remains unsatisfactory. Such filters do not selectively adsorb ionic or polar compounds from the tobacco smoke.

The Center for Applied Research, a Cambridge based research group working with the physical properties of gels, started a project in 1987 which indicated that a hydrophobic compound in the dry state, ROMAR™, could be incorporated into cigarette filters, thus removing the "tars" in cigarette smoke.

ROMAR™ possesses hydrophobic particles with externally disposed polar groups to trap and remove deleterious substances in the tobacco smoke. The hydrophobicity of the particles derives from the presence of low alkyl groups on their surfaces. The polar groups may be hydroxyl species, thiol species, or the like.

When smoked under standard conditions and to a butt length of 35 mm, the following analytical data were obtained:

TPM	10.8	mg
Tar	9.2	mg
H20	0.9	mg
Nicotine	0.8	mg
CO	11.6	mg
NOx	0.3	mg
Puff Count	9.2	
Draw	13.8	cm of H_2O

The smoking machine (see figures 2 and 3) was manufactured by the Tobacco and Health Research Institute of the University of Kentucky and designed to conform to the smoking conditions (puff volume, duration, and frequency) specified in the Coresta No. 10 procedure (Coresta Information Bulletin, September 1968). The main stream smoke was collected in a pre-weighed Cambridge filter and the crude smoke condensate (TPM) was consequently calculated as the increase in weight of the Cambridge Filter before and after smoking one cigarette.

Additional components are added for the determination of the "draw" (RTD or 'resistance to draw') and the determination of side-stream smoke TPM. The RTD for each cigarette was quantified by measuring the pressure drop, expressed in milliliters of water, under conditions defined in the Coresta No. 10 procedure. The manometer is shown in Fig. 4A. On average, each puff drew in 30 ml of smoke at a pressure of 135 mm of H_2O through the 1R4F cigarette. For analysis of the side stream smoke, Cambridge filters were added (Fig. 4B).

Commercial brand cigarettes were purchased from local vendors. The reference 1R4F cigarettes and the Cambridge filters (CM-113, 44 mm. in diameter) were

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purchased from the Tobacco and Health Research Institute of the University of Kentucky. The filters were stored, until used, in tightly sealed plastic bags. All cigarettes that were not modified were stored for at least 48 hours, in a controlled environmental unit until smoked (23-25° and 58-62% Relative Humidity). If they were modified with ROMAR™, they were returned to the proper storage conditions for at least 48 hours until smoked.

The smoking machine was turned on for at least 15 minute prior to its use. The tygon tubing was changed after every 8-10 hours of use or after 40-50 cigarettes smoked. In every experiment and trial, the unaltered 1R4F control cigarettes were smoked before experimentally modified cigarettes.

TPM REDUCTION IN MAINSTREAM SMOKE

A modified (convex or concave well) cigarette was prepared as follows. Using a sharp instrument, the filter plug was separated from the tobacco column. The filter plug was then compacted into a concave or convex well by means of a solid rod with a rounded end. The filter plug was weighed, before and after the addition of ROMAR™, on an analytical balance. The cigarette was then rejoined using an adhesive or transparent adhesive film. The transparent film allowed observation of the flow interactions of the ROMAR™ with the mainstream smoke. The smoke took the path of least resistance—at the edges. The TPM adsorbed to the ROMAR™, forming a gel.

ROMAR™ may be used in either of the two configurations shown in Fig. 5. Each of these geometries are designed to alter air flow. Configuration 5A has the best draw and seams the simplest to manufacture. Configuration 5B presents the

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smoke with a more concentrated ROMAR™ barrier and prevents ROMAR™ from impregnating the filter.

Preferably, the adsorption capacity of the filter material (2) is not affected by the heat produced during smoking. Also, it is important that the hydrophobic particles which constitute the filter material (2) be of such sizes that they cannot pass through the filter unit (1) and enter the mouth or lungs of the smoker. The amount of filter material (2) required depends on at least three factors and can be readily determined by one skilled in the art. Factors to be considered include the size of the tobacco body (3), the adsorption capacity of the filter material (2), and the amount of particulates to be removed.

The ROMAR™ was loosely packed (i.e. gravity packed and not compressed into the well.) In a second modification, the ROMAR™ was dispersed within the filter plug. Since the cigarette was never smoked to the filter plug, the complex formed between the ROMAR™, filter plug and the components of the main stream smoke can be examined (Fig. 6).

TASTE TESTS

Further tests were performed to determine the effect of ROMAR™ modified filters on cigarette taste. A questionnaire was given to 20 people selected at random and ranging in age from 20-75. Each subject was given two 1R4F cigarettes, with and without ROMAR™.

The control cigarettes was cut and taped so as to appear identical to cigarettes with ROMAR™, but was otherwise unmodified.

Four groups with 5 individuals each were selected. The groups were college students, blue-collar workers, self-employed individuals, and non-classified people.

COLORIMETRIC STUDIES

To quantify the improved removal of the "tar" component of TPM by ROMAR™, the Cambridge filters were extracted with cyclohexane. First, ten standard 1R4F cigarettes were smoked on a smoking machine, and the TPM generated was collected on a Cambridge filter. Another ten standard cigarettes were sectioned and a small amount of ROMAR™ replaced several millimeters of the filter. These cigarettes were smoked in an identical manner to the standard cigarettes and the tars collected on a Cambridge filter. A Cambridge filter blank was also prepared.

TPM REDUCTION IN SIDE-STREAM SMOKE

Limited studies were undertaken in an attempt to reduce the side-stream "TPM" that is delivered to the room and the passive smoker. A dispersion was prepared using 4%, by weight, of ROMAR™ and 96% isopropyl alcohol. The dispersion was sprayed with an atomizer onto the outer surface of the wrapping paper of twelve 1R4F cigarettes to saturation. The cigarette paper was then dried using a hair dryer. As controls, isopropyl alcohol which contained no ROMAR™ was applied to the wrapping paper of 1R4F cigarettes in the same manner.

TPM levels (mg per cigarette) were measured in the side-stream smoke collected from the cigarettes thus treated.

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Materials & Methods

EXPERIMENTAL SETUP

The effect of ROMAR™ on the levels of inhaled TPM was examined with a smoking machine, using reference 1R4F cigarettes, and Cambridge filters to collect all the TPM. TPM, designated as "tar" in the Tobacco Industry, is a brown mass composed of at least 10 hydrocarbons—particularly benzo(α)pyrene, and a small amount of moisture.

TPM ≠ tar

The 1R4F standard research cigarette, a part of the University of Kentucky's Tobacco and Health Research Institute series, is designed to yield tar and nicotine deliveries near the sales-weighted averages of those in the U.S. market. The blend for this cigarette is approximately 33.0%, 20.0%, 11.0%, and 1.0% of flue-cured, burley, Turkish and Maryland, respectively. Reconstituted sheet accounts for about 27% of the filler with 2.8% glycerine and 5.3% inverted sugar solution being added. This filtered cigarette is manufactured with an 85 mm length and 25 mm circumference.

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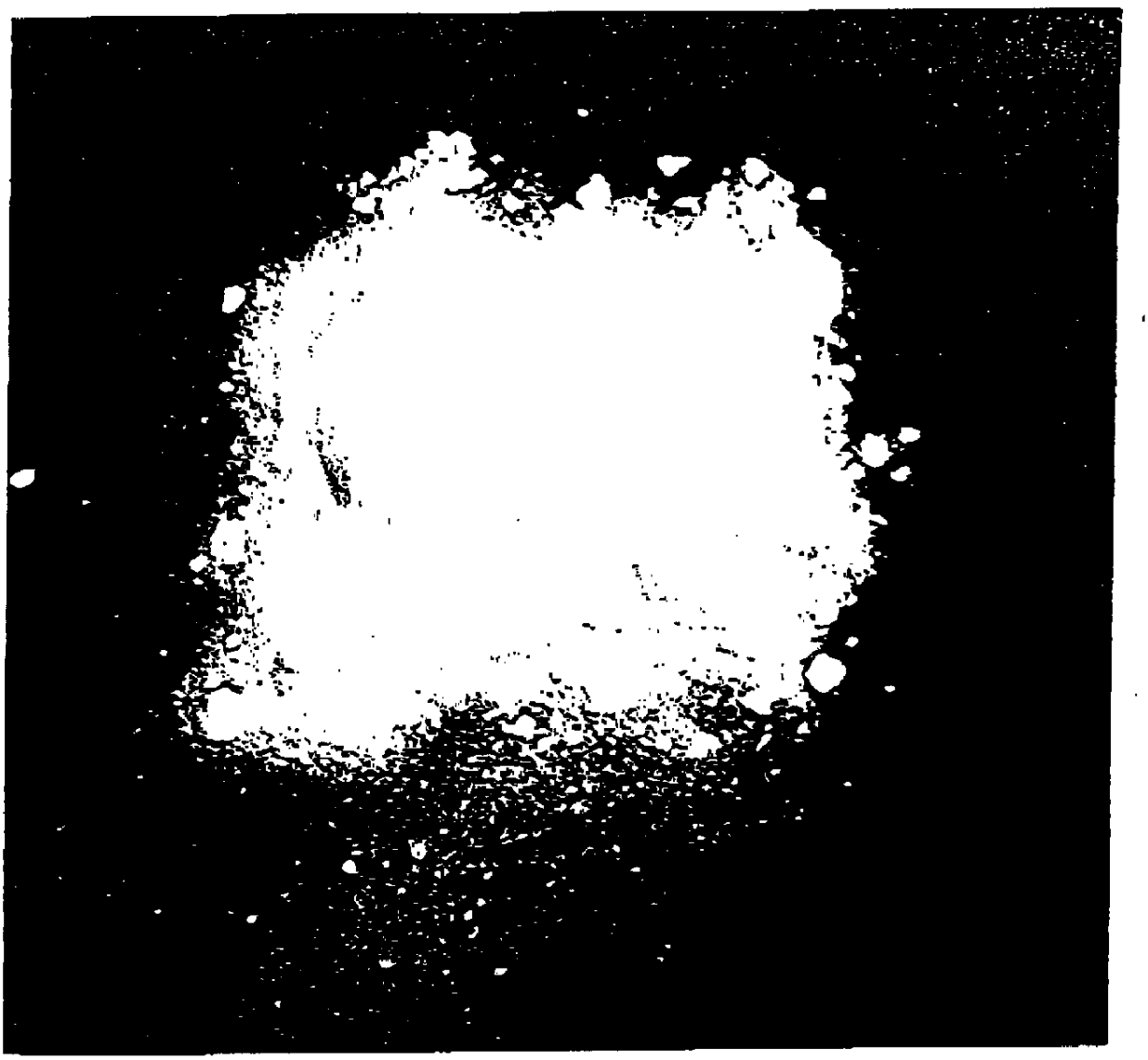

Figure 1. Photograph of ROMAR™

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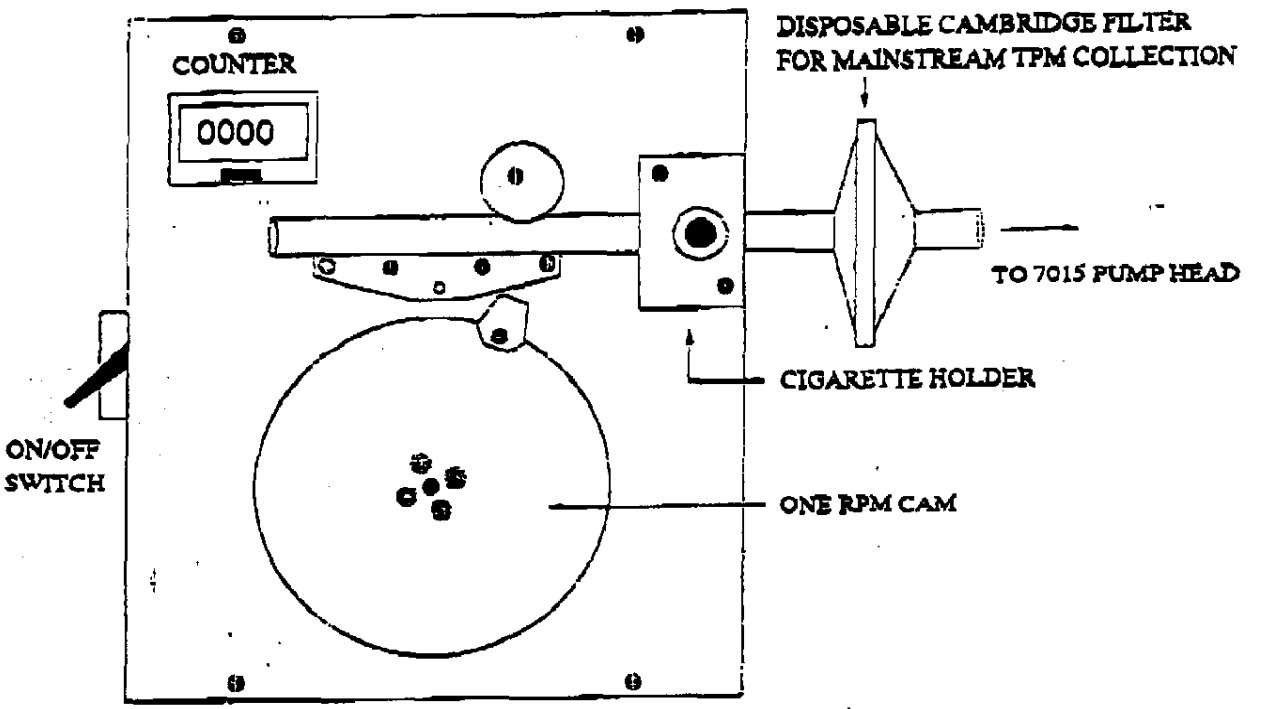

Figure 3. Schematic of the smoking machine.

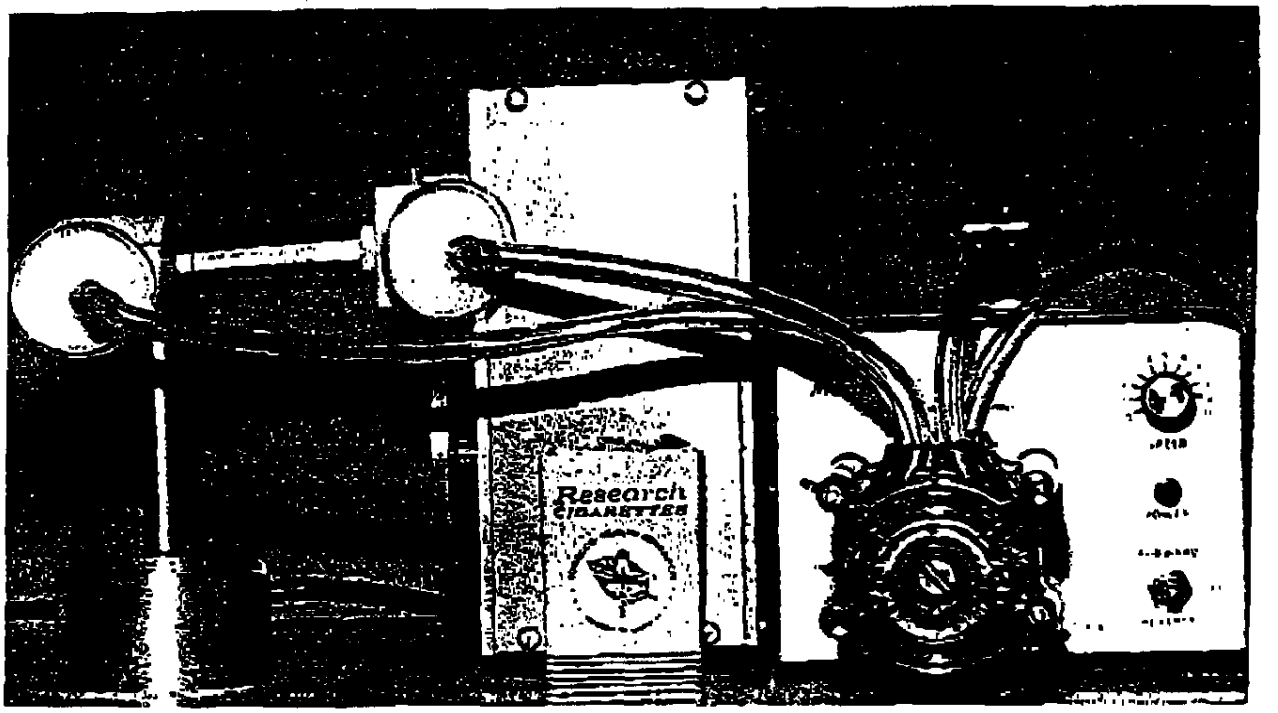

Figure 4A. Experimental setup for side-stream smoke testing. The manometer to the right is not included in this view. The Cambridge filter on the left collects the side-stream smoke.

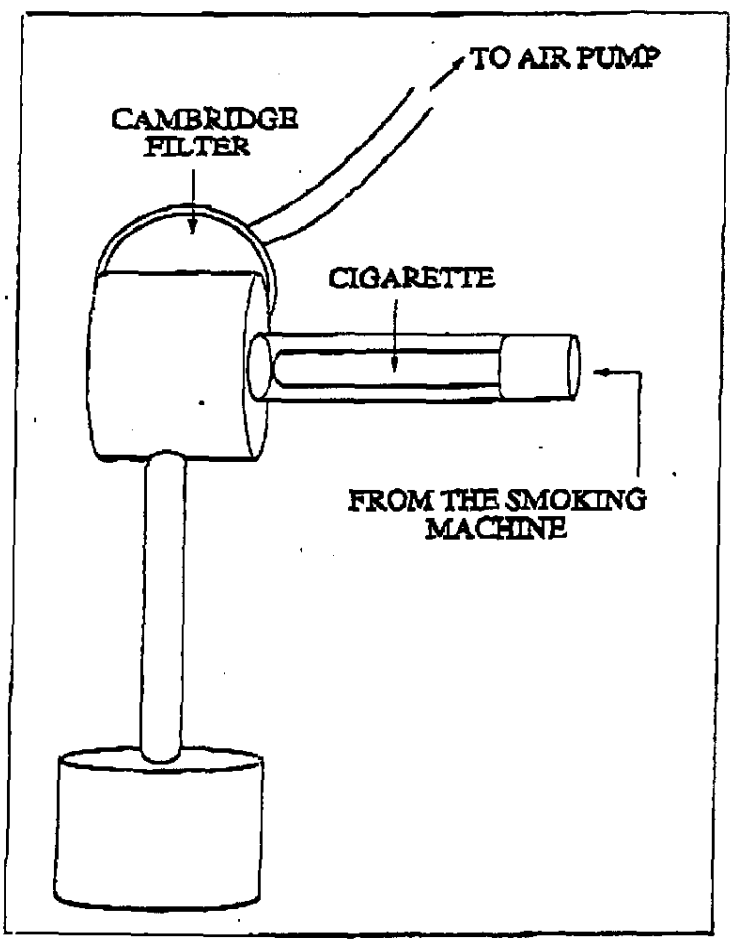

Figure 4B. Schematic of the side-stream apparatus. In this view, the Cambridge filter has been placed in the back

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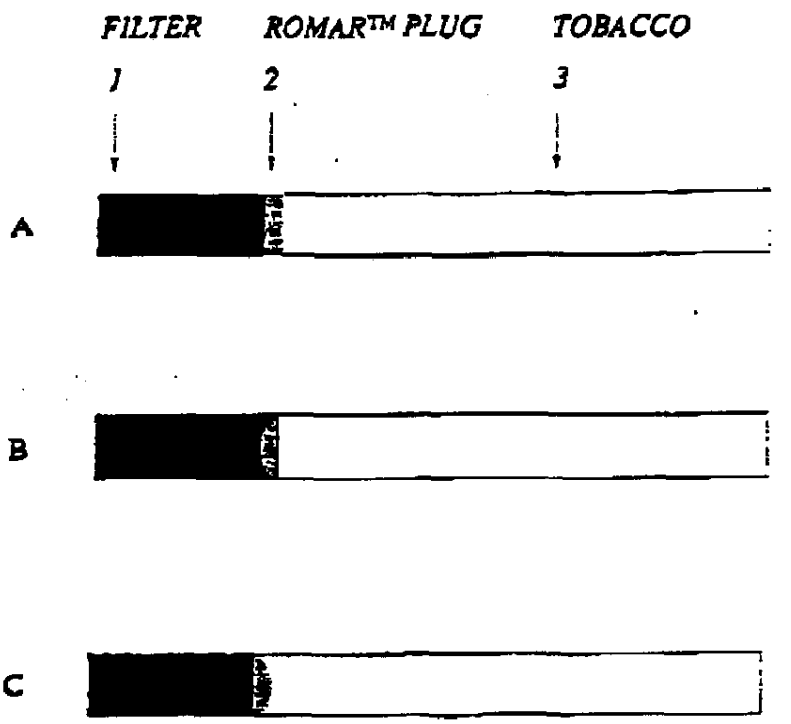

Figure 5. Cross section of three methods of using ROMAR™ in filters. In configuration A, the cylindrical plug is formed of filter fibers impregnated with ROMAR™. In config. B, the concave plug is formed of free, unmixed ROMAR™. In config. C, the convex plug is also formed of free, unmixed ROMAR™.

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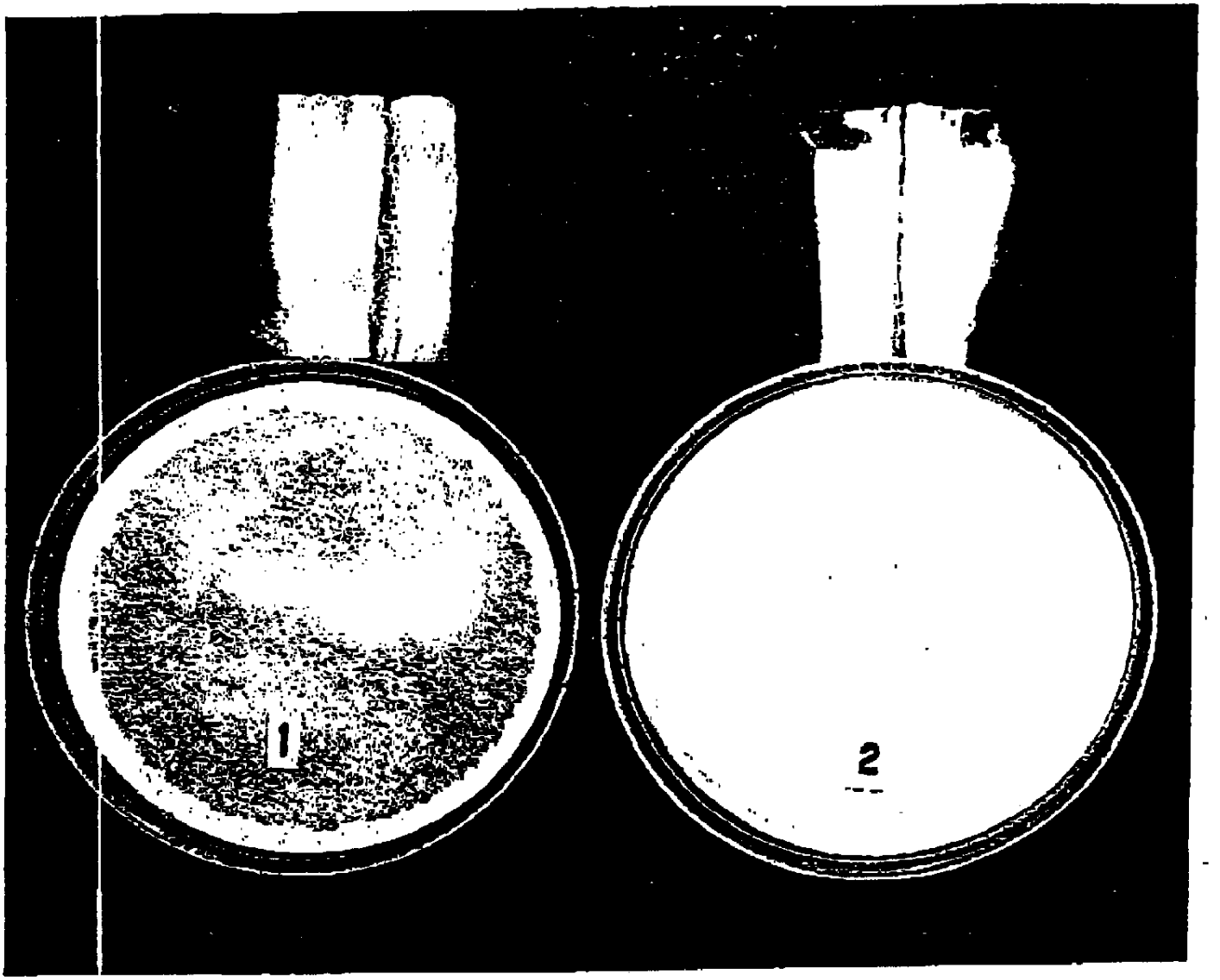

Figure 6. Cambridge filters with their respective cigarette filters. Cigarette number 1 is a control with no ROMAR™. Cigarette number 2 used a filter modified with ROMAR™. Cambridge filter 1 is accordingly darker with a larger quantity of TPM than Cambridge filter 2. The cigarette filters reveal that the majority of TPM was captured by the ROMAR™ in cigarette filter 2.

AMES ASSAY FOR MUTAGENICITY

The Ames assay employs the well-established technique of bacterial reverse mutation to screen compounds for mutagenicity.

Spencer Farr, Assistant Professor of Toxicology, Harvard School of Public Health, conducted the Ames tests for mutagenicity on the standard 1R4F cigarette. These cigarettes were run through a serial test with varying degrees of use: 2,4, and 8 puffs (the entire cigarette was considered consumed after 8 puffs) Four commercial brands of cigarettes (Marlboro Light, Vantage, True, and Carlton) were also tested with similar results. In all cases, unmodified cigarettes were compared to cigarettes with a modified pre-filter containing ROMAR™.

Salmonella typhimurium strain TA102 was obtained from Professor Bruce Ames at the University of California, Berkeley, and used in this assay. Assays were performed using a 10% rat liver S9 fraction as described in Maron and Ames (1983) with the following specific applications: Cambridge filters containing the filtrate from various amounts of cigarette smoke were immersed in 1 ml DMSO were stored at -70°C until. A total of 8 filters were tested. Fifty µl of filtrate in DMSO or DMSO alone (negative control) were mixed with 100 µl of 10% S9 fraction and 200 µl of fresh overnight culture of bacteria grown in oxoid broth. Two ml top agar was added and the culture tube was vortexed briefly before plating on VBC-histidine plates. The positive control was 5 µl and 20 µl of a 10 mg/ml mitomycin C solution. Plates were incubated at 37°C for 48 hours and colonies were scored visually. Each sample was performed in triplicate. To insure objectivity, the identity and nature of the contents of each of the 8 samples was not revealed at any time during the experiment."

"The preceding description of methods is excerpted from Spencer Farr's report commissioned by CAR. All other material excerpted from this report will be indicated by an asterisk.

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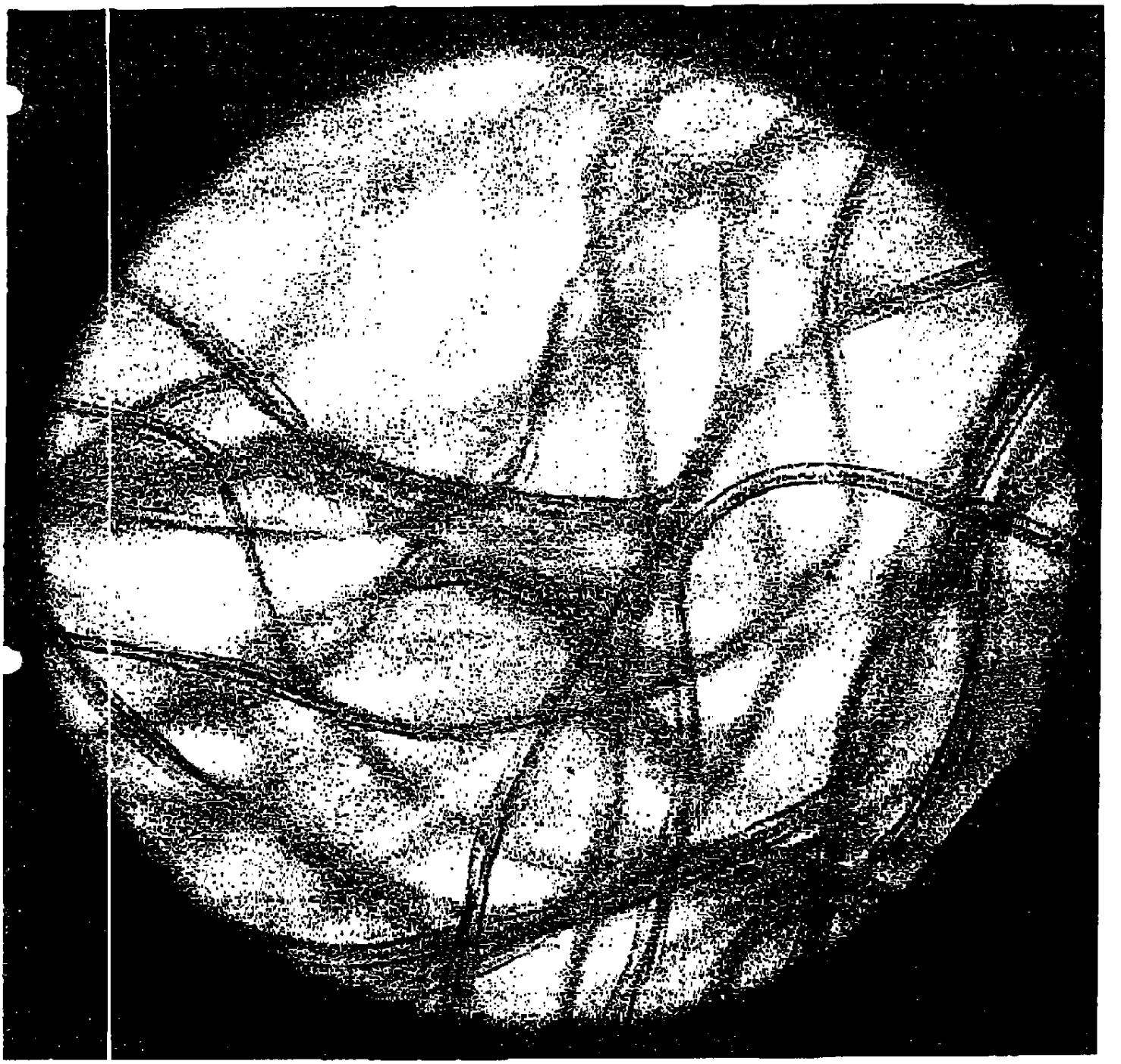

Figure 7. Photomicrograph of fibers from a clean cigarette filter

Figure 8. Photomicrograph of fibers from an unmodified cigarette filter after smoking.

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Figure 9. Photomicrograph of fibers from a cigarette filter with ROMAR™ after smoking.

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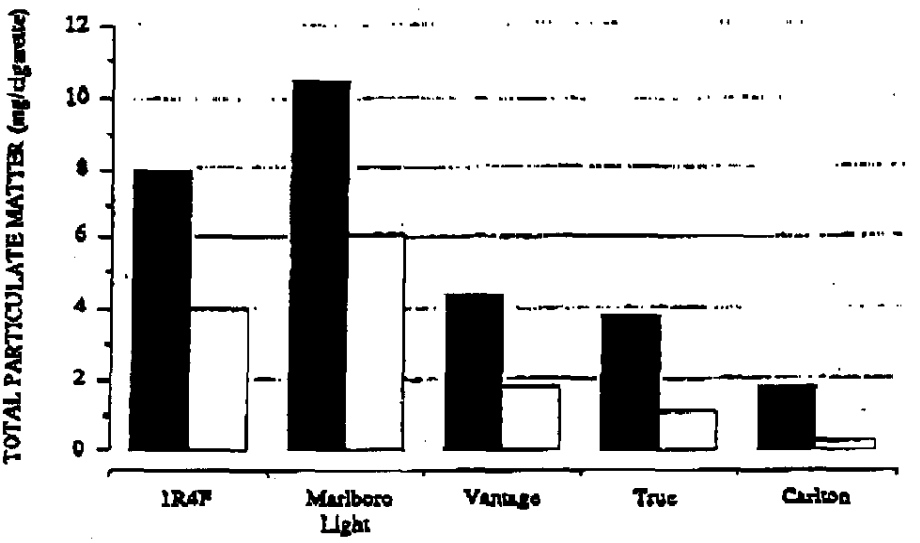

Figure 10. The effectiveness of ROMAR™ modified cigarette filters among various brands. TPM from ■ untreated and □ treated cigarettes is shown. A remarkable 90% reduction was observed in TPM removed from Carlton cigarettes. Since Carlton has by far the lowest amount of "tar" (about 1 mg/cigarette) of all the brands tested (Federal Trade Commission Report, December 1988), the ROMAR™ was required to remove only a small amount of TPM and was thus able to adsorb nearly all of it.

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Results

ROMAR™ REDUCES INHALED TPM.

Initials studies demonstrate that, upon substituting 2 mg of ROMAR™ for a small amount of the cigarette filter, a large percentage of the "TPM" was adsorbed. it was evident upon visual examination of the TPM-ROMAR™-Complex that only a small amount of the ROMAR™ had interacted. Furthermore, if one wiped the TPM-ROMAR™ plug over absorbent paper, the dark brown "TPM" was transferred to the paper. It was immediately obvious that ROMAR™ had a tremendous capacity to adsorb these cigarette "TPM's," to form what could be approximated as a gelatinous mass (see Figures 7, 8, and 9). The degree of TPM reduction for different cigarette brands is given in Fig. 10.

INFLUENCES OF FILTER DESIGN

The resistance to draw (RTD) varies with the shape of the well in which the ROMAR™ is deposited. The RTD for an unmodified 1R4F cigarette was determined to be 150 mm of H_20 (the value reported in the literature is 138 mm of H_20). Table 1 shows these results.

Table 1. The effect of ROMAR™ in different well shapes on RTD pressures of 1R4F cigarettes.

WELL SHAPE	RTD (mm of H20)	% PRESSURE INCREASE
Cylindrical	200	33
Concave	150	0
Convex	150	0

Thus with respect to RTD, modified cigarettes with a concave or convex well gave better results than those with a cylindrical well.

The resistances to draw of modified and unmodified cigarettes were also compared. Modified cigarettes were prepared with 2 mg of ROMAR™ in a concave filter as depicted in Fig. 5B.

The RTD for each cigarette was quantified by measuring the pressure drop expressed in water-column millimeters between inlet and exit of the measured cigarette when a stream of air flowed through it at 17.5 ml/second (20°C, 760 Torr). Table 2 presents these draw pressures and the % pressure increase between modified and unmodified cigarettes. With 2 mg of ROMAR™ it is possible to remove a substantial proportion of TPM without affecting draw pressures.

Table 2. Comparison of the effect of 2 mg ROMAR™ in a concave filter space on draw pressures for different brands of cigarettes. Nine puffs were used per cigarette for each brand.

BRAND	RESISTANCE TO DRAW (mm of H_2O)		% PRESSURE INCREASE
	WITH ROMAR™	*WITHOUT ROMAR™*	
Carlton	87.5	83.0	5.4
True	64.5	62.0	4.0
Vantage Lights	110.5	109.0	1.4
Marlborough Lights	155.0	156.5	0
1R4F	136.0	140.5	0

Varying the amount of ROMAR™ added changed the proportion of TPM removed without substantially changing the resistance to draw (RTD) over the range given in Table 3. As expected, the addition of more ROMAR™ resulted in the removal of more TPM.

Table 3. Comparison of the effects of varying amounts of ROMAR™ in a cylindrical filter space using 8 puffs on the standard 1R4F cigarette.

mg of ROMAR™	TPM REMOVED	RTD (mm of H_2O)
1.3	21.2%	120.5
3.0	50.0%	120.2
4.2	58.6%	120.4

These results indicate that ROMAR™ can be used to flexibly adjust TPM removal, and thus taste, without reducing draw pressure.

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TASTE TESTS

The results of the taste tests suggest that ROMAR™ does not negatively affect people's judgement of cigarette taste. In 60% of the cases, the experimental cigarette was reported to taste as good as the control, but was "smoother" and not as "harsh." The remaining 40% could not report any taste differences. No respondents found the experimental cigarettes unpleasant.

COLORIMETRIC STUDIES

Results from the colorimetric studies of TPM from Cambridge filters confirm that ROMAR™ reduces a "tar" component of TPM. The filter of the cigarette without ROMAR™ was visibly 5 to 10 times darker than the filter of the cigarette with ROMAR™ added (see Fig. 6). Following extraction, optical densities were determined (using the blank as a zero) at 363 μM/383 μM. As expected, the filter paper from the untreated cigarette yielded an optical density of 2.05 whereas the filter from the ROMAR™ modified cigarette gave a reading of only 0.62. As a first approximation, we can conclude that the ROMAR™ modified filter removed the Ligroine soluble components ("tar") of the TPM by a factor of 3.3.

ROMAR™ REDUCES TPM IN SIDE-STREAM SMOKE

The average TPM level was 13.9 mg for the ROMAR™-coated 1R4F cigarettes, and 23.7 mg for those sprayed solely with isopropyl alcohol, representing a 41% reduction. In all cases, TPM mass was reduced in the ROMAR™ treated cigarettes. This result suggests that treatment of the paper with ROMAR™ may remove a portion of the TPM contained in side-stream smoke.

These experiments with the 1R4F cigarettes were replicated 5 times. The TPM was reduced by approximately 50% and examination of the ashes showed the anticipated ROMAR™-TPM "gelatinous mass." It appears that despite the high temperature of combustion, this ROMAR™-TPM complex maintains its integrity.

ROMAR™ REDUCES THE MUTAGENS IN CIGARETTE SMOKE

At low doses, a dose-response relationship was observed between mutagenicity and the amount of TPM on the Cambridge filters. Toxicity was observed at the higher doses. Consistently, the material obtained from "prefiltered" cigarette smoke was significantly less mutagenic than unprefiltered smoke at the lower doses. Table 4 presents the results of *t*-tests which indicate that these differences are highly significant.

Table 4 . Results of Students *t*-tests (method described in Sokal and Rohlf, 1981) which demonstrate that cigarettes without ROMAR™ produce *smoke which is significantly more mutagenic.* The number of puffs indicates the degree to which the cigarette was being consumed (8 puffs approximately equals the consumption of an entire cigarette). The mean colony counts represent the number of His^+ revertants per plate above background. The probability *p* represents the probability of achieving these results through random chance alone.

NUMBER OF PUFFS	MEAN COLONY COUNT		*p*
	WITH ROMAR™	*WITHOUT ROMAR™*	
2	19 (±22)	170 (±26)	< 0.01
4	154 (±28)	383 (±12)	< 0.001
8	289 (±35)	447 (±21)	< 0.01

The probability of achieving these results through chance alone is less than 1%.

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At high doses, due to increased toxicity, no difference in mutagenicity was observed. One possible explanation for this result is that prefiltration removes a portion of the mutagenic material from the smoke, it does not remove toxic material. Further tests would be required to substantiate this hypothesis.*

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Discussion

THE REDUCTION OF TPM

Using the 1R4F reference cigarette as a standard, the filter plug was modified to obtain a filter unit that:

1. *Selectively and adjustably removes a substantial portion of TPM*
2. *Does not change the "draw."*
3. *Does not adversely affect the taste*
4. *Minimizes the amount of "tar" in side-stream smoke*

The filter unit developed to optimize these conditions consisted of a concave well formed in the filter plug and loosely (gravity) packed with approximately 2 mg of ROMAR™. The size of the well was slightly larger than the space occupied by the ROMAR™. The geometry of this well maximizes the interaction of the "TPM" with ROMAR™.

THE REDUCTION OF MUTAGENICITY

ROMAR™ significantly reduces the mutagenicity of main-stream cigarette smoke, although the actual mutation frequencies are lower than expected. There are three possible explanations for the lower frequencies:

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